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Attention: Dr Gillian Allan

25 August 2005

Madam

IN THE MATTER OF International Patent Application No. PCT/AU2004/001577 in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION entitled METHOD FOR MICROBE AND ENZYME DISCOVERY Our Ref: JSB:AJH:RMB:FP20705

We refer to the Written Opinion dated 28 February 2005 issued by the International Preliminary Examining Authority in respect of this application, and lodge herewith a Demand for International Preliminary Examination, together with the prescribed fee of $768.00.

The Examiner has objected to the presently claimed invention on the grounds that it is not novel or inventive in the light of Acha et al or Stuart et al, and that the direct measurement over time of the amount of target substrate in the vessel is a direct measure of the level of metabolism occurring in the vessel. In response, the Applicant has amended claim 1 to more clearly define the metabolism indicator used in the invention. Specifically, claim 1 has been amended to specify that the indicator of metabolism is not the test substrate. Support for this amendment can be found on page 3, lines 8 to 10 of the specification. The measurement of an indicator of metabolism that is not the test substrate as an indicator of microbial enrichment/evolution provides a tool for the discovery of useful microorganisms and enzymes.

Claim 1 of the present application requires the features of:

- *enriching* a microorganism able to metabolise a test substrate (and/or enriching an enzyme...);
- by providing a population of microorganisms (i.e. a broader class than the enriched population);
- feeding fluid into the vessel, which includes the test substrate;
- producing a signal indicative of the level of a *metabolism indicator* which represents cellular activity other than direct measurement of the change in the level of the test substrate over the time-frame of the enrichment; and

- providing an output based on the signal to enable assessment of the enrichment taking place.

The Applicant's detailed comments on the differences between the presently claimed invention and the cited prior art follows:

D1 – Acha et al

Enrichment

The Examiner states that Acha et al "*discloses adaptation (i.e. enrichment) of a biological consortium to a mixture of TCE, PCE, CT and HCB in a bioreactor.*" This is in fact not the case. According to the first sentence on page 43 of Acha et al, the microbial consortium is "previously adapted to perform extensive reductive dechlorination". Then, on page 44 first sentence, it is said that this microbial consortium is "carrier-supported". This suggests that there is no enrichment of a microorganism as the microorganism had already been enriched and there is no ability for non-performing microorganisms to be flushed through the system. At no stage was the ATR-FTIR used to monitor the actual enrichment or selection of the consortium used to inoculate the bioreactor (after immobilisation via entrapment in polyurethane foam). The on-line monitoring technique was used as an indicator of the success of bioremediation of chlorinated compounds not an enrichment or microbial/enzyme discovery process (i.e. was used to evaluate the performance of the bioreactor). Specifically, the technique was used to measure biomass or substrate utilisation. Accordingly, not only is there no relevant "enrichment" in the prior art, but there is no output that enables assessment of the enrichment taking place – the final requirement of the claim.

Furthermore, the term "enrichment" is used by Acha et al to describe absorption of the chlorinated compounds into the polymer coating thereby facilitating detection. The use of the term enrichment in this context is vastly different to the context used in the patent [eg (i) from the abstract "*The sensor consisted of an ATR internal reflection element (IRE) coated with an extracting polymer which was continuously enriched with the toxic compounds* (ii) "*no time enough to enrich the coating of the polymer*" (iii) "*this effect is the distinct difference in the enrichment behaviour of these substances as reflected in the distribution co-efficients ($f_{p/w}$) between polymer coating and aqueous solution*"). The quotes from Acha et al describe a detection methodology *not* the enrichment of microorganisms as claimed in the present application.

Metabolism indicator

Regarding the issue of whether or not a signal is produced that is indicative of the level of a metabolism indicator over time, the Examiner argues that the direct measurement of the amount of target substrate in the vessel over time is a direct measure of the level of metabolism occurring the vessel. Claim 1 has been amended to specify in step (c) that a signal is produced indicative of the level of a "metabolism indicator which represents cellular

activity other than direct measurement of the change in level of test substrate". In contrast, Acha et al measure the level of TCE, PCE, CT, and HCB in a dechlorinating bioreactor. These compounds are the test substrates provided to the methanogenic microbial community.

Therefore, Acha et al determine the concentration of the test substrates over time as a measure of the level of metabolism occurring in the bioreactor. This is different from the present invention, in which the level of a metabolism indicator represents cellular activity other than direct measurement of the change in the level of the test substrate is determined.

The technique described by Acha et al does not have the universal applicability of the method claimed in the present application. As the method of the present invention is not limited to an assessment of the level of particular metabolised substrate, it is not limited to the specific substrate metabolised. Rather, the level of a common metabolism indicator, such as O_2, can be measured. Thus the present invention is widely applicable to determining the selective enrichment for a microorganism able to metabolise a test substrate. In Acha et al, the detection of chlorinated compounds was also limited by the diffusion rate of the compound through the polymer coating on the surface of the detection system. One of the chlorinated compounds (HCB) used in the experiment could not be detected by FTIR after a 125 minute exposure time (i.e. is not a true reflection of real-time detection). The diffusion process is dependent on the type of analyte and sudden changes in the concentration of compounds that diffuse slowly may clearly not be detected by ATR-FTIR.

Moreover, the method of Acha et al only works because the amount of the test substrate, the chlorinated compounds, can be measured using FTIR techniques. If another test substrate was desired to be monitored, this would not necessarily be possible with FTIR techniques. In contrast, the method of the present invention enables assessment of the enrichment of the microbial population irrespective of the identity of the test substrate. Accordingly, even complex test substrates, such as olive oil (see Example 5 of the specification) can be assessed using the present invention.

Therefore amended claim 1 of the present application and all claims dependent thereon are novel and inventive in the light of Acha et al. because Acha et al do not disclose or suggest the use of a metabolism indicator which represents cellular activity other than direct measurement of the change in the level of the test substrate to determine enrichment of a microorganism able to metabolise the test substrate.

D2 - Stuart et al

Enrichment

The Examiner states that Stuart et al disclose "*enrichment of a dechlorinating bacterial population metabolising PCP in a computer monitored/feedback controlled continuous flow bioreactor*". This is not correct; Stuart et al used a "*two reactor system*". The first reactor is a 10 litre chemostat that is used to maintain an enriched population of PCP-degrading

microorganisms – no monitoring of this system is described. More importantly, a separate 2.5 litre reactor is then used to perform *kinetic studies* using the PCP-degrading population present in the 10 litre reactor. The 2.5 litre "*pseudo-batch*" reactor is inoculated with material taken from the 10 litre continuous reactor. The second 2.5 litre reactor had the electrodes and monitoring/feedback systems cited by the Examiner – pH, sulphide, redox potential and feedback control between pH and acetate addition. Therefore the series of electrodes and feedback systems that have been identified by the Examiner are not connected in any way to the enrichment culture. In the batch reactor a change in pH was used to control acetate addition (feedback loop).

Producing a signal indicative of the level of a metabolism indicator which represents cellular activity other than direct measurement of the change in the level of the test substrate over the time-frame of the enrichment

Stuart et al disclose a method for evaluating the growth of a culture that metabolises PCP and acetate. In effect, there are two "target compounds" in this method – the PCP and the acetate. Two techniques are used to determine the status of the metabolism over time – with regard to acetate, it is stated that the pH of the culture increases, and in response to this there was an addition of small volumes of acidic acid/acetate buffer. The Examiner argues that the pH measurement and feedback controlled maintenance of pH is a metabolism indicator, and that this is measured on-line. The Applicant respectfully submits that this argument of the Examiner is not sound. Firstly, there is no indication that the level of pH is actually provided *as an output* to enable the assessment of the selective enrichment of the microorganism that is occurring in the vessel. Secondly, the method of Stuart et al requires liquid samples to be withdrawn from the batch reactor to measure for chlorophenol and acetate content. This is an off-line technique for measuring the amount of the test substrates. Without actual measurement of the change in the level of PCP over time, it is not possible to assess in this system whether or not the microorganism being enriched is one that is metabolising the acetate only, or the PCP.

The use of a pH change as an indicator of metabolism is dependent upon the production of metabolites that affect the pH of the medium. Not all metabolic products result in pH changes in the medium (for example the production of 2,3-butanediol, ethanol and other solvents) and therefore such a technique does not have the universal application of the method claimed in the present application. In contrast, the presently claimed invention specifies a method of *enriching* for a microorganism that metabolises a test substrate, determined by an assessment of the level of a *metabolism indicator* which is not the test substrate. Therefore the method is not limited to the specific substrate metabolised. Rather, the level of common metabolism indicator, such as O_2, can be measured. Thus the present invention is widely applicable to determining the selective enrichment for a microorganism able to metabolise a test substrate.

Therefore amended claim 1 of the present application and all claims dependent thereon are novel and inventive in the light of Stuart et al. because Stuart et al do not disclose or suggest a method for enriching a microorganism able to metabolise a test substrate, nor do Stuart et al

disclose or suggest the production of a signal indicative of the level of a metabolism indicator which represents cellular activity other than direct measurement of the change in the level of the test substrate over the time-frame of the enrichment.

D3 and D4

Documents D3 and D4 disclose commercially available bioreactors, with control modules, but with no information that would enable them to be used in the methods claimed in the present application. Specifically these documents do not disclose a method for selectively enriching for a microorganism able to metabolise a test substrate and/or the enrichment of an enzyme involved in the metabolism of the test substrate.

Therefore, none of the D1 to D4 disclose or suggest a method for selectively enriching for a microorganism able to metabolise a test substrate, and/or the enrichment of an enzyme involved in the metabolism of the test substrate, as claimed in the present invention. Accordingly, the presently claimed invention is both novel and inventive in the light of the cited prior art documents.

Favourable reconsideration is requested.

Yours faithfully

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in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
entitled METHOD FOR MICROBE AND ENZYME DISCOVERY
Your Ref: TW7483/WO
Our Ref: JSB:AJH:RMB:FP20705

FIRST STATEMENT OF PROPOSED AMENDMENTS

1. Pages 3 and 63	Cancel these pages and substitute therefor replacement pages 3 and 63 lodged herewith.

GRIFFITH HACK